Bioorganic & Medicinal Chemistry Letters 20 (2010) 2456–2460

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Antimicrobial selaginellin derivatives from *Selaginella pulvinata*

Yuan Cao [a], Ji-Jun Chen [b], Ning-Hua Tan [b], Lukas Oberer [c], Trixie Wagner [c], Yong-Ping Wu [d], Guang-Zhi Zeng [b], He Yan [b], Qiang Wang [a,*]

[a] Department of Chinese Materia Medica Analysis, China Pharmaceutical University, No. 24, Tongjia Lane, Nanjing 210009, People's Republic of China
[b] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China
[c] Novartis Institute of Biomedical Research (NIBR), 4056 Basel, Switzerland
[d] Jiangsu Provincial Institute of Materia Medica, Nanjing 210009, People's Republic of China

ARTICLE INFO

Article history:
Received 5 December 2009
Accepted 4 March 2010
Available online 7 March 2010

Keywords:
Selaginella pulvinata
Selaginellaceae
Selaginellin D–F
Antimicrobial activity

ABSTRACT

Six selaginellin derivatives, including three new analogues selaginellins D–F (**1–3**), were isolated from the EtOAc extract of the whole plant of *Selaginella pulvinata* (Hook. et Grev.) Maxim. Their structures were determined on the basis of extensive physical and chemical evidence. Compounds **1** and **4** demonstrated antifungal activities against *Candida albicans*; compounds **4–6** exhibited significant antibacterial activity against *Staphylococcus aureus*.

Selaginella pulvinata (Hook. et Grev.) Maxim. (Selaginellaceae), a perennial herb widely distributed in China, is one of the two qualified species listed in Chinese Pharmacopoeia.[1] The use of this plant in traditional Chinese medicine is well-documented.[2] A literature survey showed that *Selaginella tarmariscina* contained selaginellin derivatives that are rarely found in nature,[3] which prompted us to undertake a phytochemical investigation on *S. pulvinata*, whose clinical usage and appearance are similar with those of *S. tarmariscina* as herbal medicine in China. As a result, six selaginellin derivatives (**1–6**) were isolated from its EtOAc extract, including three new analogues (**1–3**).

Selaginellin derivatives with unique acetylene bond and *p*-quinone methide functionalities, represent a rare group of naturally occurring phenolic compounds. Their natural occurrence is hitherto confined to the genus *Selaginella*. However, only four of selaginellins have thus far been identified.[3–5] The structure of the first selaginellin was confirmed based on the X-ray crystallography of its *O*-methyl derivative.[4] No report is available concerning their biological activities yet. We herein, report the isolation and structure elucidation of these new selaginellin derivatives, as well as their antimicrobial activity.

Selaginellin D (**1**) {$[\alpha]_D^{23}$ 0 (*c* 0.011, MeOH)} was obtained as needle-shaped red crystals from methanol. Its HR-ESIMS exhibited an $[M+Na]^+$ ion at *m/z* 563.1810 (calcd *m/z* 563.1834), corresponding to a molecular formula of $C_{36}H_{28}O_5$, with 23 degrees of unsaturation. The UV spectrum (265, 300, 422 nm) suggested the presence of a conjugated system. The IR spectrum showed absorption bands for hydroxyl (3431 cm^{-1}), alkynyl (2198 cm^{-1}), conjugated carbonyl (1626 cm^{-1}), and aromatic functionalities (1595, 1513 cm^{-1}). The ^1H NMR spectra data (Table 1) indicated the presence of an aromatic AB-spin system (J_{AB} = 8.4 Hz,) originating from the A-ring, and two AA′BB′ systems (J_{AB} = 8.4 Hz for both), representing the respective *p*-substituted B- and E-ring protons, as well as a four-proton multiplet at δ_H 6.80 for D-ring protons. Differentiation between the above spin systems, were effected via ^1H–^1H COSY spectrum. Protons resonated as an ABMN system with HMBC correlations (H-3, H-5/C-1; H-2, H-6/C-4) reminiscent of one semi-quinone unit (C). HMBC correlations (H-3, H-5/C-7; H-8, H-12/C-7) indicated that both C- and D-rings were connected via C-7 (δ_C 158.7). ^{13}C (Table 2) NMR spectra again disclosed the presence of an acetylene bond (δ_C 83.8 and 98.7), which was connected to B-ring as evidenced by HMBC correlations (H-28, H-32/C-27) (Fig. 2). Substitution of a CH_2OH [δ_H 4.79 (2H, d, *J* = 5.3 Hz, H-34), δ_H 5.49 (1H, t, *J* = 5.3 Hz, OH)] group at C-15 (A) was confirmed using a combination of HMBC and ROSEY experiments (Fig. 2). HMBC correlations (H-20, H-24/C-18; H-17/C-25) showed that E-ring was connected to A-ring at C-18. NOE association between H-34 and H-28 suggested the connectivity between C-26 (C≡C) and C-14. Thus C-7 could only be connected to A-ring at C-19. The above structural features suggested the skeleton of a selaginellin derivative. The extra 28 units in the MS compared to **5** revealed the presence of an additional ethyl group. Moreover, HMBC (H-35/C-10) and ROESY correlations (H-35/H-9, H-11) permitted the location of the *O*-ethyl

* Corresponding author. Tel.: +86 25 83271390; fax: +86 25 85301528.
E-mail address: qwang1949@126.com (Q. Wang).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.03.016

Table 1
^{1}H NMR data of compounds **1**–**3**[a] (δ in ppm, J in Hz)

Position	**1**[b]	**2**[c]	**2a**[c]	**2b**[c]	**3**[d]
2	6.37 (dd, 10.0, 2.0)	*	6.35 (dd, 9.6, 2.0)	6.39 (dd, 10.0, 2.0)	6.39 (dd, 10.0, 2.0)
3	7.44 (dd, 10.0, 2.8)	*	7.51 (dd, 10.0, 2.4)	7.56 (dd, 10.0, 2.8)	7.50 (dd, 10.0, 2.0)
5	7.19 (dd, 10.0, 2.4)	*	7.34 (dd, 10.0, 2.4)	7.42 (dd, 10.0, 2.8)	7.22 (dd, 10.0, 2.0)
6	6.31 (dd, 10.0, 2.0)	*	6.32 (dd, 9.6, 2.0)	6.34 (dd , 10.0, 2.0	6.32 (dd, 10.0, 2.0)
8/12	6.80 (m)	*	6.865 (d, 8.8)	6.90 (d, 9.2)	6.79 (d, 8.0)
9/11	6.80 (m)	*	6.771 (d, 8.8)	6.779 (d, 8.8)	6.67 (d, 8.0)
16	7.68 (d, 8.4)	7.73 (d, 8.0)	7.74 (d, 8.0)	8.01 (d, 8.0)	7.71 (d, 8.0)
17	7.34 (d, 8.0)	7.36 (d, 8.1)	7.38 (d, 8.0)	7.52 (d, 8.0)	7.38 (d, 8.0)
20/24	6.78 (d, 8.4)	6.86 (d, 8.5)	6.94 (d, 8.8)	7.01 (d, 8.8)	6.83 (d, 8.0)
21/23	6.54 (d, 8.4)	6.64 (d, 8.5)	6.74 (d, 8.8)	6.79 (d, 8.8)	6.58 (d, 8.0)
28/32	6.96 (d, 8.4)	7.10 (d, 8.5)	7.18 (d, 8.8)	7.27 (d, 9.2)	6.83 (d, 8.0)
29/31	6.65 (d, 8.4)	6.73 (d, 8.5)	6.84 (d, 8.8)	6.86 (d, 9.2)	6.60 (d, 8.0)
34	4.79 (d, 5.3) (2H)	5.85(s)	5.87 (s) (1H)	10.71 (s)	5.45(s) (2H)
35/39	3.96 (q, 6.9) (2H)	3.47 (s) (3H)	—	—	7.20 (d, 8.0)
36/36	1.26 (t, 6.9) (3H)	3.47(s) (3H)	—	—	7.95 (d, 8.0)

[a] Assignments confirmed by DEPT-135, HMQC, HMBC, COSY, and ROESY NMR experiments.
[b] Measured in CD_3OD at 400 MHz.
[c] Measured in acetone-d_6 at 500 MHz.
[d] Measured after refrigeration in DMSO-d_6 at 600 MHz.
* Unassignable due to broad or invisible signals.

group at C-10. Thus, the planar structure of **1** was determined as shown in Figure 1.

The stereochemistry of **1** was deduced from X-ray crystallography.[6] Due to the hindered rotation around the C-7–C-19 bond, compound **1** exists in two atropisomeric forms, as presented in the crystal structure (Figs. 3 and 4) and also evidenced by its optical rotation value. Thus, the structure of **1** was established as (*R*,*S*)-4-{(4-etho-xyphenyl)[4′-hydroxy-4-(hydroxymethyl)-3-((4-hydroxyphenyl)ethynyl)-biphenyl-2-yl]methylene}cyclohexa-2,5-dienone.

Table 2
^{13}C NMR data of compounds **1**–**3** (δ in ppm)[a]

Position	**1**[b]	**2**[c]	**2a**	**2b**[c]	**3**[d]
1	185.8	*	186.6	186.6	185.7
2	128.2	*	129.4	129.5	128.1
3	138.5	*	138.9	138.8	138.6
4	130.9	*	131.8	132.2	130.1
5	139.8	*	140.2	140.0	139.7
6	127.9	*	129.1	129.3	127.8
7	158.7	159.1	158.1	156.6	158.7
8/12	132.8	*	133.6	133.5	133.2
9/11	114.1	*	114.2	114.2	115.2
10	159.8	*	161.7	161.8	159.4
13	140.4	*	131.4	131.4	129.0
14	121.0	124.1	123.9	128.3	123.4
15	142.5	139.5	139.8	135.2	136.2
16	127.2	127.9	127.9	128.5	129.2
17	129.7	130.2	130.4	131.1	129.8
18	140.1	143.6	143.2	148.6	142.1
19	130.6	142.1	142.1	143.3	140.7
20/24	129.7	130.7	130.7	130.6	129.6
21/23	114.8	115.6	114.3	115.0	114.8
22	156.6	157.7	160.0	160.6	156.7
25	130.5	132.3	133.2	132.6	130.3
26	83.8	84.9	83.6	85.3	83.8
27	98.7	99.5	99.1	101.5	98.9
28/32	132.9	133.9	133.9	134.2	132.9
29/31	115.7	116.4	114.9	114.4	115.6
30	158.3	159.0	161.1	161.5	158.3
33	112.4	114.4	114.6	115.4	111.9
34	61.4	103.7	103.6	191.7	68.4
35/39	63.4	54.6	—	—	114.7
36/38	14.5	54.6	—	—	131.5
37/40	—	—	—	—	162.1
41	—	—	—	—	187.1

[a] Assignments confirmed by DEPT-135, HMQC, HMBC, COSY, and ROSEY NMR experiments.
[b] Measured in CD_3OD at 100 MHz.
[c] Measured in acetone-d_6 at 125 MHz.
[d] Measured after refrigeration in DMSO-d_6 at 150 MHz.
* Unassignable due to broad or invisible signals.

Selaginellin E (**2**) {$[\alpha]_D^{23}$ 0 (c 0.011, MeOH)} was obtained as needle-shaped red crystals from methanol. The UV and IR spectra exhibited typical patterns of selaginellins. The ^{1}H NMR data of **2** revealed structural features similar to those of **4**, except for the appearance of a sharp six-proton singlet at δ_H 3.47 due to a pair of magnetically equivalent *O*-methyl protons and a downfield shift of the H-34 ($\Delta\delta$ 0.91), suggesting that CH_2OH at C-15 (**4**) was replaced by CH $(OCH_3)_2$ (**2**). Confirmatory evidences were similarly available from HMBC (H-35, H-36/C-34; H-34/C-35, C-36) and ROESY correlations (H-35, H-36/H-34). The above structural deduction was in accordance with the HR-ESIMS (*m*/*z* 555.1780 $[M-H]^-$) analyses, with 44 mass units more than that of **4**.

The structure of **2** could be assigned as 34-dimethoxyselaginellin, but the interpretation of the spectra was very problematic due to severe line broadening of either C- or D-ring, some of them even appearing to be obscured in base-line. This contrasts notably with **1**, which displayed well dispersed signals of corresponding protons and carbons. Such confusing spectra of **2** probably derived from the tautomeric equilibrium due to integrated effects of the hindered rotation around C-7–C-19 bond and the delocalization between C- and D-rings, as shown in Scheme 1. We assumed that the distinct difference in the NMR spectra of **1** and **2** could be explained in terms of the effect of the different substitution, and the alkylation of C-10-OH of selaginellins could block the tautomerism via blockage of the delocalization. To testify this presumption, we synthesized the *O*-methyl derivative of **2**. As expected, all formerly broadened signals got sharpened and well-resolved. Nevertheless, even though the HR-ESIMS exhibited a single ion at *m*/*z* 599.2444 $[M+H]^+$, compatible with the molecular formula $C_{39}H_{34}O_6$ for the desired derivative, duplication of most ^{1}H and ^{13}C NMR resonances suggested that the product was a mixture of two compounds, **2a** and **2b** in a 1:0.95 ratio, as determined by the integration of some well-resolved ^{1}H NMR resonances for each compound. Unfortunately, efforts to separate this mixture were unsuccessful due to insufficient material. Therefore, the structure elucidation of **2a** and **2b** was performed on the mixture. The signals at δ_H 10.71 (s) and δ_C 191.7 revealed the presence of an aldehyde group, which could be ascribable to **2b**, formed by hydrolysis from **2a** under acidic condition (Scheme 2). Further 2D NMR

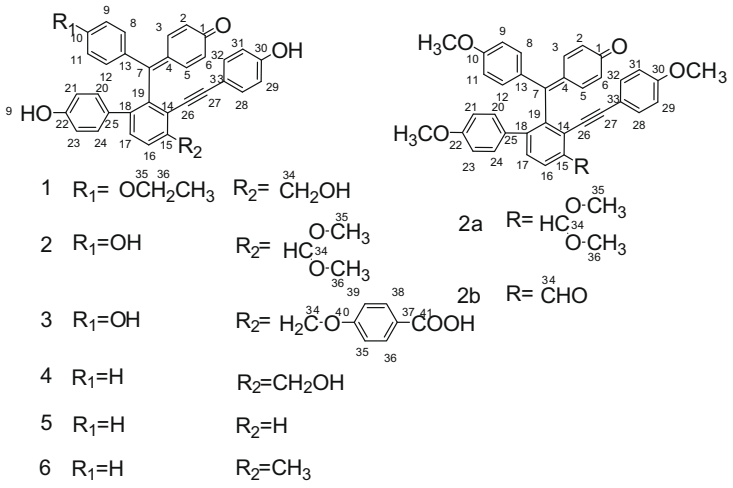


Figure 1. Structures of compounds **1**–6.

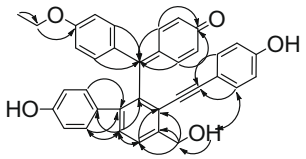


Figure 2. Key HMBC and ROESY correlations of compound **1**.

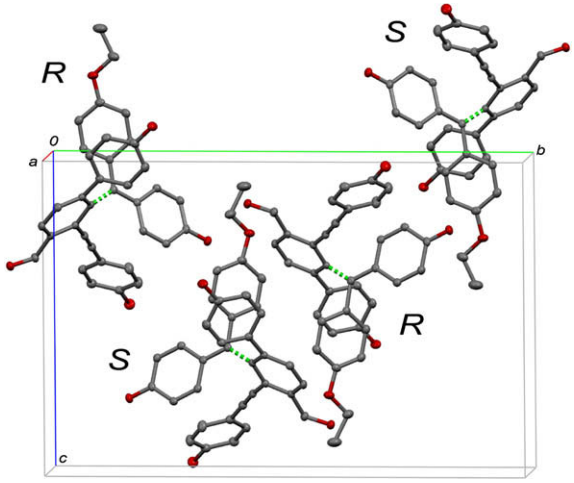


Figure 3. Packing of compound **1** in the crystal. View down *a*. Hydrogen atoms and water molecules omitted for clarity. Chiral axes visualized by dotted bonds.

experiments (COSY, HSQC, HMBC, and ROESY) helped to the full assignments of **2a** and **2b** (Fig. 1).

Compound **2** presumably adopted the same stereochemistry as **1**, likewise originating from the hindered rotation around the C-7–C-19 bond. Thus, its structure was elucidated as (*R*,*S*)-4-{(4-hydroxyphenyl)[4′-hydroxy- 4-(dimethoxy-methyl)-3-((4-hydroxyphenyl) ethynyl)biphenyl-2-yl]methylene}cyclohexa-2,5-dienone(Fig. 1). We could, however, find no evidence for the existence of such a compound in the original extract. Consequently, we speculated that compound **2** most likely represented an artificial product of its aldehydic precursor formed by acidic methylation conditions during chromatographic separation on silica gel.

Selaginellin F (**3**) {$[\alpha]_D^{23}$ 0 (*c* 0.013, MeOH)} red solid, was determined to have a molecular formula of $C_{41}H_{28}O_7$ by the HR-ESIMS. The close structural relationship between compounds **4** and **3** was evident from similar spectral features. The most significant difference between the ^{1}H NMR spectra of compounds **4** and **3** was the replacement of a hydroxy group by the *p*-substituted benzoic acid moiety in that of **3**.

However, pertinent challenge still remained for **3**, how to produce well-resolved spectra, when chemical derivatization was impossible due to limited amount available. Sarma et al.[7] studied the effects of solvents on the delocalization of diaryl quinone methides, and proposed that the polarity and hydrogen-bonding abilities of the solvents were key factors involved: DMSO-d_6 would resulted in a highly delocalized system, making the aromatic protons attached to phenol and quinone rings chemically equivalent, but in the case of acetone-d_6, it would lead to less delocalization, rendering those aromatic protons inequivalent. However, the spectra of selaginellin A and B measured in acetone-d_6 (Cheng et al., 2008), as well as that of selaginellin C in DMSO-d_6 (Tan et al., 2009), all reflected the phenomena of extensive delocalization. We also observed in ^{1}H NMR of **3** (in acetone-d_6), the protons of either C- or D-ring merged into a broad signal. This indicates that solvent is not necessarily the only reason responsible for this process. By accident, we found that after lyophilisation, the re-measured spectra (in DMSO-d_6) were notably free of the adverse effects of tautomerism (Tables 1 and 2). Thus, it could be rationalized that the amount of water in sample had a substantial effect on the appearance of the NMR spectra. An increased water amount might enhance the speed of the tautomerism, as evidenced by signal anomalies, and the same comes true to the corresponding carbon peaks (see Supplementary data). On the contrary, the equilibrium might be slowed down by reducing water amount present in the sample after lyophilisation, hence culminating in the unequivocal NMR spectra. Similarly, its optical activity reflected the existence of two atropisomers for compound **3**, emanating from the restricted rotation at C-7–C-9 bond. Thus, **3** was elucidated as (*R*,*S*)-4-{(4′-hydroxy-2-[(4-hydroxylphenyl)(4-oxocyclo-hexa-2,5-dienylidene)methyl]-3-[(4-hydroxy-phenyl)eth-ynyl)biphenyl-4-yl]methoxy}benzoic acid.

A comparison of the spectroscopic and physical data with those published allowed us to establish the structures of the known compounds as selaginellin (**5**),[4] selaginellin A (**6**), selaginellin B (**7**).[3] So far, all reported selaginellins exist in two atropisomeric forms.

Compounds **1**, **4**–**6** were evaluated for antimicrobial activity against *Staphylococcus aureus* and *Candida albicans*, with the results presented in Table 3. Compounds **4**–**6** showed significant inhibitory activity against *S. aureus* (IC_{50} 4.9, 1.2 and 1.2 μg/mL, respectively); compounds **1** and **4** possessed good antifungal activity

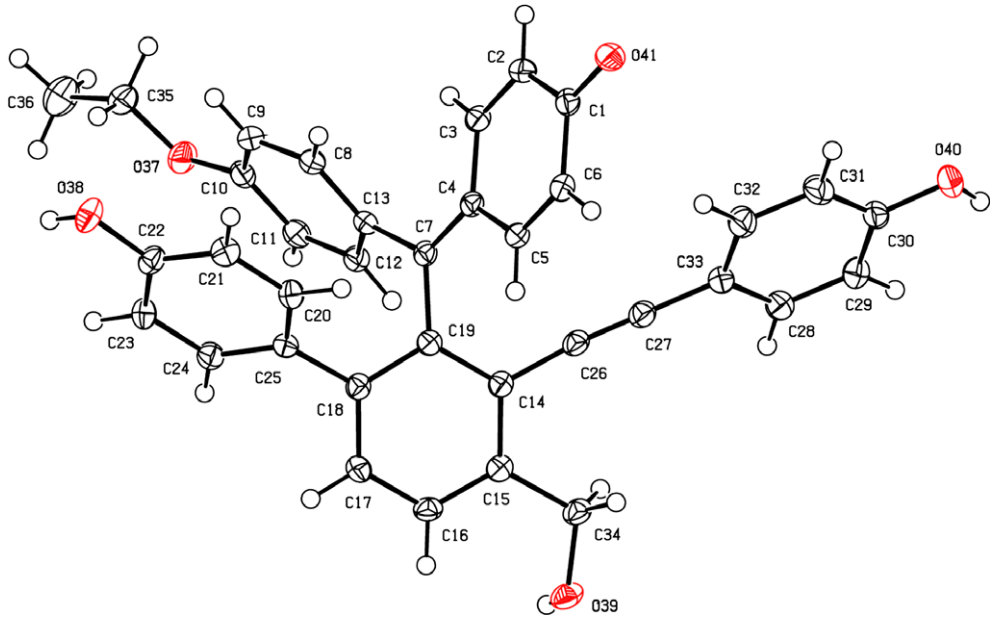
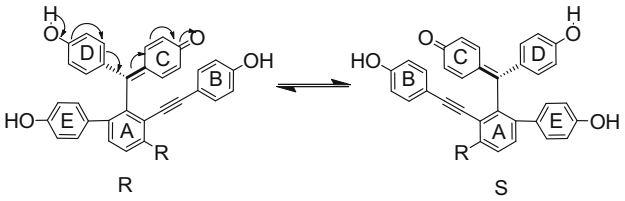


Figure 4. Structure of compound (*R*)-**1** in the crystal. Ellipsoids drawn at 50% probability, hydrogen atoms with arbitrary radii.



Scheme 1. The tautomerism of compounds **2** and **3**.

against *C. albicans* (IC_{50} 5.3 and <3.1 μg/mL, respectively) (Table 3). Interestingly, compounds **4**–**6**, with the hydroxy group at C-10, were active towards *S. aureus*, whereas, compound **1** with the *O*-ethyl substituent, was inactive. This indicated that the hydroxy group at C-10 might be important for the antibacterial activity. These results encourage us to continue our research of this series by synthesizing additional selaginellin derivatives for structure–activity relationship study, with the aim of obtaining compounds that are more potent and selective towards bacteria or fungus.

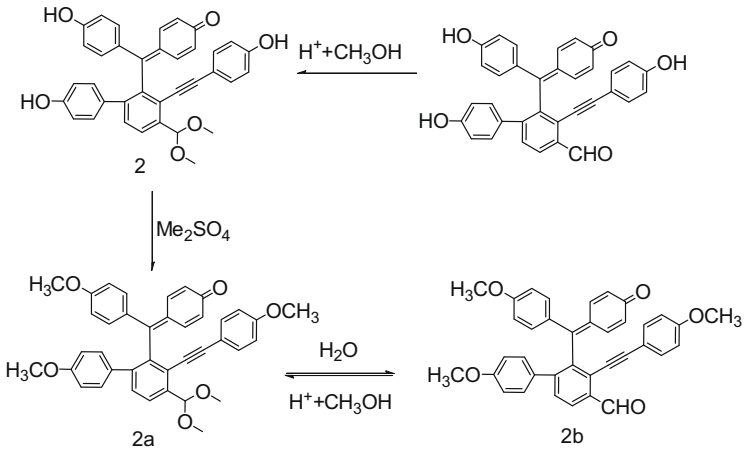
Table 3
Antimicrobial activity of some isolates from *S. pulvinata* (IC_{50} and MIC in μg/mL)

Compound	*C. albicans*[a]	*S. aureus*	
		IC_{50}[a]	MIC[b]
1	5.3	na	na
4	<3.1	4.9	9.6
5	na	1.2	4.6
6	na	1.2	2.6
Ampicillin		0.1	0.84
Miconazole nitrate salt	<1.2		

na = not active.
[a] IC_{50} = the test concentration that affords 50% inhibition of growth.
[b] MIC = the test concentration that affords 99% inhibition of growth.

Acknowledgments

We thank Dr. Z. Y. Jiang, Ms X. M. Zhang, Dr. Y. M. Zhang (State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany), and Dr. J. S. Wang (Depart-



Scheme 2. Formation of compounds **2**, **2a**, and **2b**.

ment of Natural Medicinal Chemistry, China Pharmaceutical University) for technical assistant, Dr. C. Guenat (Novartis Pharma AG) for recording the HRESI mass spectra and Mr. P. Piechon (Novartis Pharma AG) for collecting the X-ray data. We are also indebted to Mr. M. X. Lai (Guangxi Academy of Chinese Medicine and Pharmaceutical Science) for collecting plant material.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.016.

References and notes

1. Chinese Pharmacopoeia Commission, Pharmacopoeia of the People's Republic of China, Vol. I, People's Medical Publishing House, Beijing, 2005, pp 154.
2. Jiangsu New Medical College, Zhong Yao Da Ci Dian, Shanghai Science and Technology Press, Shanghai, 2004, pp 1472–1473.
3. Cheng, X. L.; Ma, S. C.; Yu, J. D.; Yang, S. Y.; Xiao, X. Y.; Hu, J. Y.; Lu, Y.; Shaw, P. C.; But, P. P. H.; Lin, R. C. *Chem. Pharm. Bull.* **2008**, *56*, 982.
4. Zhang, L. P.; Liang, Y. M.; Wei, X. C.; Cheng, D. L. *J. Org. Chem.* **2007**, *72*, 3921.
5. Tan, G. S.; Xu, K. P.; Li, F. S.; Wang, C. J.; Li, T. Y.; Hu, C. P.; Shen, J.; Zhou, Y. J.; Li, Y. J. *J. Asian Nat. Prod. Res.* **2009**, *11*, 1001.
6. *X-ray crystallographic analysis of* **1**: A red platelet from methanol, size 0.09·0.07·0.02 mm^3, $C_{36}H_{30}O_6$ (selaginellin D·1H_2O), M_r = 558.60, monoclinic, space group $P2_1/c$ (No. 14) with a = 8.258(2), b = 23.325(6), c = 15.455(4) Å, β = 96.281(13)°, V = 2959.0(13) $Å^3$, Z = 4, D_c = 1.254 g cm^{-3}, 62399 reflections measured, 5222 independent (R_{int} = 0.0505), 3.44° < θ < 66.62°, T = 100 K, 391 parameters, no restraints, R_1 = 0.0341, wR_2 = 0.0813 for 4428 reflections with $I > 2$ (I), R_1 = 0.0435, wR_2 = 0.0866 for all 5222 data, GoF = 1.044, res. el. dens. = +0.16/−0.21 e $Å^{-3}$. The structure was solved by dual-space recycling methods and refined on F^2 with the SHELXTL program suite. Non-hydrogen atoms were refined anisotropically, hydrogen atoms at selaginellicin A were refined in idealized positions using a riding model, hydrogen atoms at the crystal water were refined isotropically. Crystallographic data for the structure have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 745059. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1 EZ, UK [fax: +44-(0)1223-336033 or email: deposit@ccdc.cam.ac.uk].
7. Sarma, R. J.; Kataky, R.; Baruah, J. B. *Dyes Pigms* **2007**, *74*, 88.